Appendix C1

Summary Minutes from the Peer Review Panel Meeting on May 19-21, 2009

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Summary Minutes

Independent Scientific Peer Review Panel Meeting

Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches

Consumer Product Safety Commission Headquarters
Fourth Floor Hearing Room
Bethesda Towers Building
Bethesda, MD

May 19 - 21, 2009

Peer Review Panel Members:

A. Wallace Hayes, Ph.D., DABT, FATS, ERT (Peer Review Panel Chair)	Visiting Scientist (Harvard), Harvard School of Public Health, Andover, MA; Principal Advisor, Spherix Incorporated, Bethesda, MD
Hongshik Ahn, Ph.D.	Professor, Stony Brook University, Stony Brook, NY
Paul Bailey, Ph.D.	Bailey & Associates Consulting, Neshanic Station, NJ
Richard Dubielzig, D.V.M.	Professor, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI
Henry Edelhauser, Ph.D.[1]	Professor of Ophthalmology and Director of Ophthalmic Research, Emory University School of Medicine, Atlanta, GA
Mark Evans, D.V.M., Ph.D., DACVP	Pathology Lead for Ophthalmology Therapeutic Area, Pfizer Global Research and Development at La Jolla Drug Safety Research and Development, San Diego, CA
James Jester, Ph.D.	Professor of Ophthalmology and Biomedical Engineering, Endowed Chair, University of California-Irving, Orange, CA

[1] Unable to attend the Panel meeting, but participated in the review of all materials.

Peer Review Panel Members:

Tadashi Kosaka, D.V.M., Ph.D.	Associate Director, Chief, Laboratory of Immunotoxicology and Acute Toxicology, Toxicology Division, The Institute of Environmental Toxicology, Ibaraki, Japan
Alison McLaughlin, M.Sc., DABT	Health Canada, Environmental Impact Initiative, Office of Science and Risk Management, Health Products and Food Branch, Ottawa, Ontario, Canada
J. Lynn Palmer, Ph.D.	Associate Professor, Department of Palliative Care and Rehabilitation Medicine, University of Texas, MD Anderson Cancer Center, Houston, TX
Robert Peiffer, Jr., D.V.M., Ph.D., DACVO	Senior Investigator, Merck Research Laboratories, Safety Assessment Toxicology, West Point, PA
Denise Rodeheaver, Ph.D., DABT	Assistant Director, Alcon Research Ltd., Department of Toxicology, Fort Worth, TX
Donald Sawyer, D.V.M., Ph.D., DACVA	Professor Emeritus, Retired, College of Veterinary Medicine, Michigan State University, East Lansing, MI
Kirk Tarlo, Ph.D., DABT	Scientific Director, Comparative Biology and Safety Sciences, Amgen, Inc., Thousand Oaks, CA
Daryl Thake, D.V.M., Dipl. ACVP[1]	Midwest ToxPath Sciences, Inc., Chesterfield, MO
Scheffer Tseng, M.D., Ph.D.[1]	Director, Ocular Surface (OS) Center, Medical Director OS Research & Education Foundation, Directory R&D Department, Tissue Tech, Inc., Ocular Surface Center, P.A., Miami, FL
Jan van der Valk, Ph.D.	Senior Scientist, Departments of Animals, Science and Society, Faculty of Veterinary Medicine, Utrecht University, Netherlands Centre Alternatives to Animal Use (NCA), Utrecht, Netherlands
Philippe Vanparys, Ph.D., DABT	Managing Director, CARDAM (VITO), Mol, Belgium
Maria Pilar Vinardell, Ph.D.	Director, Department of Physiology, Professor of Physiology and Pathology, Department Fisologia, Facultat de Farmacia, Universitat de Barcelona, Barcelona, Spain
Sherry Ward, Ph.D., M.B.A.	In Vitro Toxicology Consultant, BioTred Solutions, Science Advisor, International Foundation for Ethical Research, New Market, MD

Peer Review Panel Members:

Daniel Wilson, Ph.D., DABT	Mammalian Toxicology Consultant, Toxicology and Environmental Research Consulting, The Dow Chemical Company, Midland, MI
Fu-Shin Yu, Ph.D.	Director of Research, Department of Ophthalmology & Anatomy, School of Medicine, Wayne State University, Detroit, MI

ICCVAM and ICCVAM Ocular Toxicity Working Group Members:

Meta Bonner, Ph.D.	EPA, OPP, Washington, DC
Robert Bronaugh, Ph.D.	FDA, CFSAN, College Park, MD
Pertti Hakkinen	NLM, Bethesda, MD
Masih Hashim, D.V.M., Ph.D.	EPA, OPP, Washington, DC
Jodie Kulpa-Eddy, D.V.M. (ICCVAM Vice-Chair)	USDA, Riverdale, MD
Donnie Lowther	FDA, CFSAN, College Park, MD
Deborah McCall	EPA, OPP, Washington, DC
Jill Merrill, Ph.D. (OTWG Chair)	FDA, CDER, Silver Spring, MD
John Redden	EPA, OPP, Crystal City, VA
RADM William Stokes, D.V.M., DACLAM (Director, NICEATM)	NIEHS, Research Triangle Park, NC
Marilyn Wind, Ph.D., (ICCVAM Chair)	CPSC, Bethesda, MD

Invited Experts:

Rodger Curren, Ph.D.	Institute for In Vitro Sciences (IIVS), Gaithersburg, MD
Arnhild Schrage, Ph.D.	Experimental Toxicology and Ecology, BASF SE, Ludwigshafen, Germany

European Centre for the Validation of Alternative Methods, ICCVAM OTWG Liaison:

João Barroso, Ph.D.	European Centre for the Validation of Alternative Methods, Ispra, Italy

Public Attendees:

Attendee	**Affiliation**	**Day Attended**		
		1	**2**	**3**
Odelle Alexander	Syngenta Crop Protection, Greensboro, NC	√	√	√
Ian Blackwell	EPA, Antimicrobials Division, Arlington, VA	√	√	-
Krishna Deb	EPA, Antimicrobials Division, Arlington, VA	√	√	-
Noe Galvan	Clorox Services Co., Pleasanton, CA	√	√	√
Earl Goad	EPA, Antimicrobials Division, Arlington, VA	√	√	√
John Harbell	Mary Kay Inc., Addison, TX	√	√	√
Leon Johnson	EPA, Antimicrobials Division, Crystal City, VA	√	-	-
Eli Kumekpor	Invitrogen, Frederick, MD	√	-	√
Pauline McNamee	The Procter & Gamble Co., Egham, Surrey, U.K.	√	√	√
Michelle Piehl	MB Research Laboratories, Spinnerstown, PA	√	-	-
Patrick Quinn	Accord Group, Washington, DC	-	-	√
Hans Raabe	Institute for In Vitro Sciences, Gaithersburg, MD	-	√	√
Mary Richardson	Bausch & Lomb, Rochester, NY	√	√	√
Michael Rohovsky	Johnson & Johnson, New Brunswick, NJ	√	√	√
Kristie Sullivan	Physicians Committee for Responsible Medicine, Oakland, CA	-	-	√
Neil Wilcox	Consultant/FDA, College Park, MD	√	√	-

NICEATM:

RADM William Stokes, D.V.M., DACLAM	Director
Debbie McCarley	Special Assistant to the Director

Support Contract Staff— Integrated Laboratory Systems, Inc.:

David Allen, Ph.D.	Elizabeth Lipscomb, Ph.D.
Jonathan Hamm, Ph.D.	Linda Litchfield
Nelson Johnson	Greg Moyer, M.B.A.
Brett Jones, Ph.D.	James Truax, M.A.

Abbreviations used in participants' affiliations:

CDER = Center for Drug Evaluation and Research

CFSAN = Center for Food Safety and Applied Nutrition

CPSC = U.S. Consumer Product Safety Commission

ECVAM = European Centre for the Validation of Alternative Methods

EPA = U.S. Environmental Protection Agency

FDA = U.S. Food and Drug Administration

ICCVAM = Interagency Coordinating Committee on the Validation of Alternative Methods

ILS = Integrated Laboratory Systems, Inc.

NICEATM = National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

NIEHS = National Institute of Environmental Health Sciences

NLM = National Library of Medicine

OPP = Office of Pesticide Programs

OTWG = Ocular Toxicity Working Group

USDA = U.S. Department of Agriculture

TUESDAY, MAY 19, 2009

Call to Order and Introductions

Dr. Hayes (Peer Review Panel Chair) called the meeting to order at 8:30 a.m. and introduced himself. He then asked all Peer Review Panel (Panel) members to introduce themselves and to state their name and affiliation for the record. He then asked all the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) staff, the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) members, the ICCVAM Ocular Toxicity Working Group (OTWG) members, the European Centre for the Validation of Alternative Methods (ECVAM) staff person, and members of the public to introduce themselves. Dr. Hayes stated that there would be opportunities for public comments during the discussions associated with each of the ten test method topics. He asked that those individuals interested in making a comment register at the registration table and provide a written copy of their comments, if available, to NICEATM staff. Dr. Hayes emphasized that the comments would be limited to seven minutes per individual per public comment session, and that, while an individual would be welcome to make comments during each commenting period, repeating the same comments at each comment period would be inappropriate. He further stated that the meeting was being recorded and that Panel members should speak directly into the microphone.

Welcome from the ICCVAM Chair

Dr. Wind, U.S. Consumer Product Safety Commission (CPSC) and Chair of ICCVAM, welcomed everyone to CPSC and to the Panel meeting. Dr. Wind stressed the importance of this Panel's efforts, especially considering the public health importance of ocular safety testing and hazard labeling. Dr. Wind noted that approximately 125,000 home eye injuries occur each year and over 2,000 workers suffer eye injuries each day, many of which are caused by accidental exposure to chemicals or chemical products. Dr. Wind also reviewed the statutes and regulations requiring ocular testing.

Dr. Wind thanked the Panel members for giving their expertise, time, and effort and acknowledged their important role in the ICCVAM test method evaluation process. Dr. Wind also emphasized the importance of public comments that are considered by the Panel in this process and the Panel's role in the development of ICCVAM final test method recommendations.

Welcome from the Director of NICEATM, and Conflict-of-Interest Statements

Dr. Stokes, Director of NICEATM, stated the Panel meeting was being convened as a National Institutes of Health (NIH) Special Emphasis Panel and was being held in accordance with applicable U.S. Federal Advisory Committee Act regulations. As such, Dr. Stokes indicated that he would serve as the Designated Federal Official for this public meeting. He reminded the Panelists that, when they were originally selected, they had signed conflict-of-interest statements in which they identified any potential conflicts of interest. He then read the conflict-of-interest statement and again asked members of the Panel to identify any potential conflicts for the record. Dr. Hayes asked the Panel members to declare any direct or indirect conflicts based on Dr. Stokes' statements and to recuse themselves from voting on any aspect of the meeting where these conflicts were relevant.

Dr. Sawyer declared a potential conflict-of-interest regarding his employment with Minrad Inc., a company that manufactures inhalation anesthetics. Dr. Ward declared a potential conflict-of-interest regarding her consulting relationship with a company that manufactures antimicrobial cleaning products. Dr. Rodeheaver indicated that she worked for Alcon, a manufacturer of the topical anesthetics proparacaine and tetracaine. Dr. Vanparys declared a potential conflict-of-interest regarding his company's involvement in the conduct of the Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) test method.

Overview of the ICCVAM Test Method Evaluation Process

Dr. Stokes opened his presentation by thanking the Panel members for their significant commitment of time and effort preparing for and attending the meeting. He noted that this is an international Panel, made up of 22 different scientists from six different countries (Belgium, Canada, The Netherlands, Japan, Spain, and the United States). He explained that the purpose of the Panel was to conduct an independent scientific peer review of the information provided on several proposed alternative ocular safety test methods, a testing strategy, and proposed refinements to the *in vivo* rabbit eye test method. This assessment is to include an evaluation of the extent that each of the established ICCVAM criteria for validation and regulatory acceptance has been appropriately addressed for each test method or testing strategy. The Panel is then asked to comment on the extent that the available information and test method performance in terms of accuracy and reliability supports the ICCVAM draft recommendations. Dr. Stokes noted that the first ICCVAM Ocular Peer Review Panel met in 2005 to evaluate the validation status of four alternative test methods (Bovine Corneal Opacity and Permeability [BCOP], Isolated Chicken Eye [ICE], Isolated Rabbit Eye [IRE], and the HET-CAM) for their ability to identify ocular corrosives or severe irritants. The Panel recommended two of these test methods (BCOP and ICE) on a case-by-case basis for use in a tiered-testing strategy with test method-specific applicability domain restrictions. ICCVAM and the Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) endorsed the Panel's recommended use for these test methods. The Panel also recommended that, while the IRE and HET-CAM test methods were potentially useful in a tiered-testing strategy with appropriate restrictions, additional data were needed to fully assess their usefulness and limitations for regulatory testing. ICCVAM prepared a test method evaluation report (TMER) and provided a transmittal package (i.e., Panel report, SACATM and public comments, TMER and associated materials) to the ICCVAM Federal agencies for their response as required by the ICCVAM Authorization Act of 2000 (ICCVAM 2000). All Federal agencies with ocular testing requirements endorsed the BCOP and ICE test method recommendations. Dr. Stokes noted that five Panel members from the 2005 review are on the current Panel (i.e., Drs. Henry Edelhauser, A. Wallace Hayes, Robert Peiffer, Scheffer Tseng, and Philippe Vanparys).

Dr. Stokes then provided a brief overview of ICCVAM and NICEATM, and identified the 15 Federal agencies that comprise ICCVAM. He summarized the purpose and duties of ICCVAM (as described in the ICCVAM Authorization Act of 2000[2]), noting that ICCVAM, as an interagency committee, does not carry out research and development or validation studies. Instead, ICCVAM, in conjunction with NICEATM, carries out critical scientific evaluations of the results of validation studies for proposed test methods to assess their usefulness and limitations for regulatory testing, and then makes formal recommendations to ICCVAM agencies.

Dr. Stokes then described the ICCVAM test method evaluation process, emphasizing the many opportunities for stakeholder input during numerous public comment periods.

As part of this process, a working group of Federal scientists designated for the relevant toxicity testing area (e.g., the OTWG) and NICEATM prepare a draft background review document (BRD) that provides a comprehensive review of all available data and information. ICCVAM considers all of this available data and information and then develops draft test method recommendations on the proposed usefulness and limitations of the test methods, test method protocol, performance standards, and future studies. The draft BRD and the ICCVAM draft test method recommendations are made available to the Panel and the public for review and comment. The Panel reviews the draft BRD and evaluates the extent to which the established ICCVAM validation and regulatory acceptance criteria have been adequately addressed and the extent that the demonstrated accuracy and reliability support the ICCVAM draft test method recommendations. A Panel report is published and then considered, along with public and SACATM comments, by ICCVAM in developing final recommendations.

[2] http://iccvam.niehs.nih.gov/docs/about_docs/PL106545.pdf

ICCVAM forwards these final recommendations to the ICCVAM member agencies for their consideration and possible incorporation into relevant testing guidelines.

He concluded by summarizing the timeline for 2009 for the ICCVAM evaluation and peer review of the ocular test methods and approaches, including a *Federal Register* notice in March announcing the Panel meeting, the projected publication of the Panel report in July, and transmittal of ICCVAM final recommendations to Federal agencies in November.

ICCVAM Charge to the Panel

Dr. Stokes reviewed the charge to the Panel:

(1) Review the ICCVAM draft BRDs for completeness and identify any errors or omissions (e.g., other relevant publications or available data).

(2) Evaluate the information in the draft BRDs to determine the extent to which each of the applicable ICCVAM criteria for validation and regulatory acceptance of toxicological test methods have been appropriately addressed.

(3) Consider the ICCVAM draft test method recommendations for the following and comment on the extent to which they are supported by the information provided in the BRDs: proposed test method usefulness and limitations, proposed recommended standardized protocols, proposed test method performance standards, and proposed future studies.

Dr. Stokes thanked the OTWG and ICCVAM for their contributions to this project and acknowledged the contributions from the participating liaisons from ECVAM, the Japanese Center for the Validation of Alternative Methods (JaCVAM), and Health Canada. He also acknowledged the NICEATM staff for their support and assistance in organizing the Panel meeting and preparing the review materials.

Overview of the Agenda

Dr. Hayes outlined the process for reviewing each of the topics. First, the test method developer or other expert will describe the test method protocol and procedures, followed by a presentation summarizing the test method validation database and test method performance for each draft BRD or summary review document (SRD) given by a member of the NICEATM staff. An ICCVAM OTWG member will then present the ICCVAM draft test method recommendations. Following presentations, the Evaluation Group Chair responsible for the topic under consideration will present the Evaluation Group's draft recommendations and conclusions followed by Panel discussion. Public comments will then be presented followed by the opportunity for questions to the public commenters and additional Panel discussion. After consideration of the public comments, the Panel will then vote to accept the Panel consensus, with any minority opinions being so noted with a rationale for the minority opinion provided.

Draize Rabbit Eye Test and Current Ocular Regulatory Testing Requirements and Hazard Classification Schemes

Ms. McCall of the U.S. Environmental Protection Agency (EPA) presented the relevant U.S. and international statutes and regulations for ocular safety testing (e.g., EPA, CPSC, Food and Drug Administration [FDA], Occupational Safety and Health Administration [OSHA], European Union [EU], and Organisation for Economic Co-operation and Development [OECD]). She summarized the Draize scoring system for corneal, iridal, and conjunctival lesions in the rabbit, using representative photographs for reference. She also discussed optional but potentially useful assessments of ocular injury (e.g., fluorescein staining, corneal thickness, depth of corneal injury, photographic documentation, and histopathology) that are not routinely included in the Draize eye test. Ms. McCall then provided an overview of the various U.S. and international hazard classification schemes for ocular corrosivity and irritation (i.e., EPA, EU, Globally Harmonized System of Classification and

Labelling of Chemicals [GHS], and Federal Hazardous Substances Act [FHSA]). She noted that, based on the recently adopted European Union Regulation on the Classification, Labelling and Packaging of Substances and Mixtures (i.e., the CLP Regulation), the EU will move to the GHS system after December 1, 2010, for substances and after June 1, 2015, for mixtures. Ms. McCall also identified the required signal words for labeling based on each regulatory classification.

Use of Topical Anesthetics and Systemic Analgesics to Avoid or Minimize Pain and Distress in Ocular Toxicity Testing

On behalf of NICEATM, Dr. Allen reviewed the relevant sections of the draft BRD on the routine use of topical anesthetics and systemic analgesics in *in vivo* ocular irritation testing.

Dr. Merrill then presented the ICCVAM draft recommendations for the routine use of topical anesthetics and systemic analgesics in *in vivo* ocular irritation testing for the Panel to consider.

Panel Evaluation

Dr. Sawyer (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the routine use of topical anesthetics and systemic analgesics in *in vivo* ocular irritation testing and ICCVAM draft test method recommendations. Dr. Sawyer indicated that anesthetic requirements vary enormously among species. For instance, cats require approximately 40% more anesthetic than humans to achieve a similar level of anesthesia. Therefore, any protocol designed to minimize or eliminate pain needs to be individualized to the target species. The Evaluation Group proposed an alternative to the ICCVAM anesthetic/analgesic protocol to be used during all *in vivo* rabbit ocular irritation testing. Dr. Sawyer outlined the Evaluation Group's proposed protocol, which is divided into pretreatment and posttreatment regimens as follows:

Pretreatment Analgesia:
Buprenorphine 0.01 mg/kg subcutaneous (SC) (60 minutes before test substance application [TSA]). Dr. Sawyer noted that buprenorphine is classified as an opioid agonist-antagonist analgesic with a wide margin of safety in rabbits, minimal sedation, and relatively long duration. It has been found to be effective in managing pain in small animals, and is given before application of the test substance because the most effective method of managing pain and distress is to administer the analgesic preemptively to prevent establishment of central sensitization.

One or two drops of 0.5% proparacaine hydrochloride, applied to the eye three times at 5-minute intervals starting 15 minutes pre-TSA. Last application would be five minutes pre-TSA. Anticipated duration of action: 30 - 60 minutes. Dr. Sawyer stated that proparacaine is preferred because application to the eye would be less painful and the suggested application sequence is to assure effective penetration of the epithelial layer.

Eight hours post-TSA:
Buprenorphine 0.01 mg/kg SC and meloxicam 0.5 mg/kg SC. Dr. Sawyer noted that the timing is to reinforce the initial level of analgesia to carry over until the next morning (the duration of analgesia is expected to be at least 12 hours for buprenorphine and at least 24 hours for meloxicam). The combination of an opioid and a nonsteroidal anti-inflammatory drug (NSAID) such as meloxicam is a well-tested approach to balanced analgesia. Used for post-operative or chronic pain in dogs since 1997, meloxicam has been found to have effective application in rabbits.

Day two through day seven post-TSA:
Buprenorphine 0.01 mg/kg SC every 12 hours and meloxicam 0.5 mg/kg SC every 24 hours. Dr. Sawyer noted that buprenorphine and meloxicam should be continued for seven days post-TSA unless signs of ocular injury sufficient to cause pain and discomfort appear. If so, this systemic analgesic protocol would continue until the test is completed.

Rescue Analgesia:
Dr. Sawyer also outlined a procedure where, if a test subject shows signs of physical pain or discomfort during the test interval using the above protocol, a rescue dose of buprenorphine at 0.03 mg/kg SC could be given as needed every eight hours instead of 0.01 mg/kg SC every 12 hours. Meloxicam would continue with the same dose and interval.

Dr. Sawyer pointed out that buprenorphine and meloxicam were synergistic and have an excellent safety profile in clinical practice. A question was raised concerning the interval of dosing throughout the test period and the burden that it would impose on the testing laboratory. The Panel agreed that a ±30-minute interval is appropriate for the administration of the systemic analgesics.

Dr. Dubielzig indicated that the impact of the NSAID on inflammatory aspects of the Draize rabbit eye test is unknown, but the Panel did not consider such affects to be limited and therefore not likely to be a problem. Dr. Jester questioned the need to continue analgesic treatment through day seven when Category III or IV substances would have cleared by day three. He suggested an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approach where treatment is continued through day four. Dr. Peiffer suggested that the temporal aspect be removed and that treatment be continued only if there are signs of discomfort. **The Panel agreed that treatment should be stopped after day four (instead of day 7, as suggested above) if there are no signs of discomfort**. The Panel agreed that pain assessment should be made and recorded daily.

Dr. Jester raised a concern that the use of preservatives in the topical anesthetics may interfere with the irritation response. The Panel agreed that the use of preservative-free proparacaine should be required. Dr. Stokes asked how long after the administration of the systemic analgesics a rescue dose can be administered. Dr. Sawyer indicated that, due to the wide margin of safety, the rescue dose can be given immediately afterward if necessary.

Dr. Jester expressed concern that dilution of the test substance could occur if a significant amount of liquid anesthetic remained in the eye. Dr. Peiffer indicated that, in his experience, the 5-minute interval is reasonable and should not pose a problem for test substance dilution.

In response to the evaluation guidance question specific to testing situations where the use of topical anesthetics would be considered inappropriate, the Panel indicated that drugs to be used for ocular effects, such as eye drops, need to be tested by other means. However, the focus of this evaluation is eye irritation hazard classification; therefore, the proposal would be relevant to all such testing. The Panel did not know of additional systemic analgesics that might have greater efficacy in relieving ophthalmic pain associated with chemically-induced injuries. The Panel also agreed that there were no additional pain-related chemically-induced injuries to the eye that the proposed alternate analgesic proposal would not adequately address.

The Panel expressed general concern about the use of transdermal patches to deliver anesthetics due to the need for shaving prior to patch application and the possibility of skin irritation. In addition, with multiple applications, the availability of irritation-free skin sites may pose a problem. Most importantly, analgesic patches have proven to be unreliable in clinical practice with significant animal-to-animal variation as well as species-to-species variation when comparing effectiveness and duration of effect. The Panel also indicated a greater concern about self-mutilation due to severe pain during eye irritation testing than about the potential for the systemic analgesics to alter the ocular injury response. Dr. Jester indicated that there was insufficient information in the BRD to make this assessment.

The majority of the Panel agreed that the tetracaine information provided in the ICCVAM BRD could be applied to other topical anesthetics such as proparacaine. Dr. Ward indicated that additional studies on cell proliferation, migration, and cytotoxicity could be done with topical anesthetics to provide some assurance that they behave in a manner similar to tetracaine. Although it was previously noted

that anesthetic/analgesic use was for all *in vivo* eye irritation tests, the Panel indicated that administration of post-application analgesics is not a concern if a standard dosing regimen is used throughout and not adjusted for each animal to avoid overdosing side effects.

The Panel also agreed that the clinical signs of post-application pain and distress are adequately described and that no other clinical signs should be added. In the event of an eye infection, the Panel agreed that secondary treatment should be considered, the signs and symptoms of the eye infection should be documented, and the animal should be immediately removed from the study. Finally, the Panel agreed that all relevant data had been adequately considered in the BRD.

The Panel considered its proposal to be more appropriate than the ICCVAM-proposed recommendations in terms of the type and frequency of dosing for topical anesthetics and systemic analgesics. The Panel agreed with the ICCVAM draft recommendations for future studies. Therefore, it recommended refinement of the current *in vivo* test system to evaluate ocular irritation utilizing contemporary/novel technologies to address both concerns. The Panel recommended the following:

- New animal studies should only be considered when absolutely necessary in developing new strategies for testing.
- Products that are overpredicted when anesthetic and analgesic pretreatment is used should be identified.
- Animal responses should be collected in tests currently being conducted to determine whether refinements are warranted in the dosing and timing of anesthetic, analgesic, and antibiotic treatments.
- Rabbit ocular specimens should be submitted for histopathological evaluation to develop an archive of specimens.
- Digital photographs of lesions/observations should be collected.
- Analysis of the variability in rabbit wound-healing responses would help determine whether or not it is due to variability in the ocular defense linking to the neuroanatomic integration.
- Studies should be conducted to determine whether the timing and dosing of systemic analgesics with topical anesthetics might alter the ocular defense enough to change the classification of test substances.
- Cytology samples from the surface of the eye should be collected.
- Studies should be conducted to investigate the appropriateness of using proparacaine instead of tetracaine.
- Studies should be conducted to evaluate the impact of using the NSAID meloxicam with buprenorphine.
- New technologies (e.g., new imaging modalities and quantitative/mechanistic endpoints) should be incorporated into the Draize rabbit eye test, refining/changing it to make it a more humane test that is also more reliable.

Public Comments

No public comments were made.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion with one abstention,

Dr. Rodeheaver, who cited a potential conflict-of-interest due to her employment by a manufacturer of anesthetic products.

Use of Humane Endpoints in *In Vivo* Ocular Irritation Testing

On behalf of NICEATM, Dr. Allen reviewed the relevant sections of the draft BRD on the use of humane endpoints in *in vivo* ocular irritation testing for the Panel.

Dr. Merrill then presented the ICCVAM draft recommendations for the use of humane endpoints in *in vivo* ocular irritation testing for the Panel to consider.

Panel Evaluation

Dr. Sawyer (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the use of humane endpoints in *in vivo* ocular irritation testing and ICCVAM draft test method recommendations. The Panel agreed that each of the current and proposed humane endpoints detailed in the BRD are sufficiently predictive of irreversible or severe effects (i.e., GHS Category 1, U.S. EPA Category I, EU R41) that they should be used routinely as humane endpoints to terminate a study as soon as they are observed. The Panel also agreed that animals should be observed at least once per day (at least twice daily for the first three days) to ensure that termination decisions are made in a timely manner. The Panel agreed that there was insufficient data in the BRD to determine the adequacy of pannus as a recommended humane endpoint. The Panel also agreed that the use of fluorescein staining was an appropriate technique for evaluating eye injury; however, the technique needs to be better described before a reasonable conclusion regarding its value can be made. Dr. Jester suggested that the use of fluorescein staining had not been adequately discussed in this BRD.

The Panel emphasized that, in some cases, decisions to terminate a study should be based on more than one endpoint. Very severe endpoints (e.g., corneal perforation) would be adequate alone to terminate a study. Other biomarkers considered useful by the Panel as routine humane endpoints included extent of epithelial loss, limbal ischemia, and/or stromal loss, and depth of corneal damage.

In response to the question regarding other earlier biomarkers/criteria indicative that painful lesions can be expected to fully reverse, the Panel indicated eyes with conjunctival scores without corneal/iris scores would be expected to recover. The Panel indicated that the destruction of 50% of the limbus will result in pannus in rabbits and, therefore, the ICCVAM draft recommendation requiring 75% for early termination may be excessive. In addition, the Panel indicated that the humane endpoints described in the BRD were sufficient to ensure that the lesions would not reverse. The Panel did agree that the available data and information supported the ICCVAM draft recommendations on humane endpoints. The Panel recommended that studies be developed to identify better and earlier endpoints, such as those seen with fluorescein staining, and that these endpoints should be incorporated into current testing guidelines.

Public Comments

No public comments were made.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion.

Adjournment

Dr. Hayes adjourned the Panel for the day at 5:45 p.m., to reconvene at 8:30 a.m. on Wednesday, May 20, 2009.

WEDNESDAY, MAY 20, 2009

Dr. Hayes called the meeting to order at 8:28 a.m. and asked Dr. Stokes to discuss the conflict-of-interest for the day's planned topics. Dr. Stokes read the conflict-of-interest statement and Dr. Hayes asked the Panel to declare any conflicts-of-interest. The conflicts-of-interest declared by Panel members on day one of the meeting were repeated.

Dr. Hayes then asked for introductions from the Panel, NICEATM staff, members of ICCVAM and the OTWG, and those in attendance for the public session.

HET-CAM Test Method

Dr. Schrage reviewed the various HET-CAM test method protocols (i.e., IS[A], IS[B], S-Score, Q-Score, and IT) and BASF experience with the test method. Dr. Schrage stressed the need for harmonization of HET-CAM protocols, endpoints, and scoring methods. BASF has conducted a retrospective review of 145 test substances, including a broad variety of chemicals and formulations, which revealed that overall accuracy, false positive rates, and false negative rates were not acceptable. The specificity and sensitivity were especially affected by solubility in both water and oil. These data were submitted to the journal Alternatives to Laboratory Animals in April 2009. Dr. Schrage said she would be willing to share the HET-CAM data on these 145 substances with NICEATM following publication.

Dr. Vanparys said that he would be willing to provide NICEATM with HET-CAM data using the IS(B) analysis method to determine if conversion to the IS(A) method was feasible. He added that, in his experience, the HET-CAM test method can be sensitive for the identification of substances not labeled as irritants.

On behalf of NICEATM, Dr. Allen reviewed the HET-CAM draft BRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the HET-CAM test method for the Panel to consider.

Panel Evaluation

Dr. Wilson (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the HET-CAM test method and ICCVAM draft test method recommendations. He noted that HET-CAM classified four EPA Category III substances incorrectly as Category IV (i.e., they were false negative in HET-CAM). However, he said that regulators would be more concerned if the false negative substances were EPA Category I or Category II. Some Panelists did not consider these substances likely to be a significant risk. Dr. Stokes suggested adding a statement defining an acceptable rate for false positives and false negatives. Dr. Wilson expressed concern that, while three of the four animals had an EPA Category III classification that cleared in seven days, one animal had a conjunctival redness score of two that cleared to one in seven days but required 14 days to completely resolve (i.e., return to a score of zero). Such lesions would not be considered inconsequential.

The Panel discussed the low number of mild and moderate substances used in the performance analyses, and that additional substances in these categories would be needed before a conclusion on the usefulness of HET-CAM could definitively be reached. The Panel also recognized that the validation database does not include substances currently regulated by EPA and that collection of additional data is needed. Therefore, given the limited data for mild and moderate substances, the Panel did not support the ICCVAM draft test method recommendation for use of the HET-CAM to identify substances not labeled as irritants from all other classes.

Dr. Peiffer said that he was concerned with the recommendation to test increasing concentrations of test substances. He stated that while dose-response curves are preferred for scientific studies, they are

not practical for regulatory testing. Dr. Sawyer agreed that increasing concentrations should not be a requirement. Ms. McLaughlin argued that use of different concentrations allows the investigator to see if increasing the concentration affects the outcome. She stated that poor predictivity might result from use of a concentration that produces an ineffectual or weak response, whereas the comparative effect of a higher concentration would provide useful information. The Panel agreed to remove the concentration requirement from the test method protocol but to include it as a general recommendation for additional research.

Ms. McLaughlin offered a minority opinion with respect to the Panel's recommendation on the use of the HET-CAM test method to identify substances not labeled as irritants from all other classes. Ms. McLaughlin stressed that personal care products are not regulated in the U.S. as they are in Europe and Canada. Ms. McLaughlin stated that the HET-CAM test method could be used as an alternative to the Draize rabbit eye test to evaluate personal care products in situations where they are regulated. Dr. Hayes asked Ms. McLaughlin to write a short paragraph to note the rationale for her opposition to the majority view for inclusion in the Panel report. Ms. McLaughlin drafted the following text:

Based on the demonstrated performance as outlined in the ICCVAM draft recommendations, HET-CAM can be used to screen not labeled as irritants from other irritant categories for the restricted applicability domain (surfactant-based formulations and oil/water emulsions). The rationale for this dissenting view is based on the fact that there were 60 substances in the overall database. The hazard category distribution was: 25 Category I; 2 Category II; 18 Category III; and 15 Category IV. The sensitivity of HET-CAM is 91% (41/45), resulting in a false negative rate of 9% (4/45). Among the four false negatives for the EPA system, 100% (4/4, all oil/water emulsion cosmetic formulations) were EPA Category III substances based on conjunctival redness score of two that required at least three days to resolve. The lesions noted *in vivo* indicated mild ocular irritation and are unlikely to represent a significant hazard. As such, the HET-CAM could be considered useful as a screening test for EPA Category IV substances not labeled as irritants from all other categories for the restricted applicability domain of surfactant-based formulations and oil/water emulsions. The sensitivity for GHS and EU was high enough for each system to warrant HET-CAM test method use (i.e., 100% sensitivity; 31/31 and 26/26, respectively for GHS and EU [from the ICCVAM draft BRD, Tables 6-2 and 6-12]) also with domain restriction. This performance demonstrates that HET-CAM could be used to screen EU or GHS hazard not labeled as irritant classifications from other irritant categories for the restricted applicability domain of surfactant-based formulations and oil/water emulsions. It should be noted that, for regulatory purposes, sensitivity (the proportion of all positive substances that are classified as positive) is most important from a public health perspective and the HET-CAM performed well in this regard.

The Panel discussed the ICCVAM draft recommended protocol for the HET-CAM test method. Dr. Vinardell said that she would like to see a statement added to the protocol to wash out any leftover solids after 30 seconds (as currently recommended in the EU Annex V). Dr. Hayes asked Dr. Vinardell to provide a statement for Dr. Wilson to include in the Panel report.

The Panel discussed the HET-CAM test method performance. One Panelist suggested that a Chi-square analysis should be included to ensure that differences in classification were statistically significant. Dr. Ahn was asked if a power analysis could be used to determine if the number of substances in the mild and moderate classification was adequate to differentiate the irritant classifications. Dr. Ahn said that there should be at least three substances in each classification category to conduct a power analysis.

The Panel discussed the need for Good Laboratory Practice (GLP) studies. Dr. Hayes emphasized that a study is either GLP compliant or it is not. He said that the phrase "spirit of GLP" should not be used in the Panel report. He also said that the term "original data" should be used rather than "raw data."

The Panel agreed that data from studies not conducted under GLP guidelines could be used to increase knowledge about the applicability domain of a test method but that laboratories should provide sufficient detail about the conduct of the study to understand any deviations from GLP guidelines.

The Panel discussed additional sources of HET-CAM data to expand the applicability domain and the number of mild and moderate substances tested. Dr. Allen noted that Dr. Debbasch, a principal contact for data acquisition, had left L'Oreal. Dr. Hayes said that *cosmeceuticals* represented a gray zone between cosmetics and personal-care formulations, and this class of products should be considered. Ms. McLaughlin said that the inclusion of a single ingredient (e.g., a UV-blocking material) could change the regulatory requirements for a formulation from an unregulated personal care product to a regulated material in Canada. She said that the applicability domain and database used in the ICCVAM draft BRD should be adequate to warrant use of the HET-CAM test method for personal care products that are not labeled as irritants. The Panel did not support the use of additional studies to identify the full range of irritation but supported additional studies to identify substances not labeled as irritants from all other classifications.

Public Comments

Dr. Barroso from ECVAM commented that the false negatives using the EPA classification system, which are substances not labeled as irritants using the GHS classification system, result because the EPA classification system categorizes substances based upon the most severe category observed among the test rabbits (i.e., not based on the majority classification among rabbits tested). Dr. Barroso also said that because the types of formulations regulated by EPA are not present in the database that the EPA classification system should not be given too much weight.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted to approve the recommendations as revised during the discussion with one minority opinion, Ms. McLaughlin, and one abstention, Dr. Vanparys, who cited a potential conflict-of-interest with the HET-CAM test method, which he had worked on at Johnson & Johnson.

Isolated Chicken Eye Test Method

On behalf of NICEATM, Dr. Allen presented an overview of the ICE test method protocol and reviewed the ICE draft BRD. One Panelist asked why the test method was limited to three eyes. Dr. Allen explained that the incubation apparatus contained 10 chambers, sufficient for three groups of three eyes and a negative control. However, the ICCVAM ICE test method protocol, upon which the recently submitted OECD Test Guideline is based, includes both positive and negative controls.

Dr. Jester said that the term fluorescein *staining* should be used rather than *retention*. He also asked how the EPA classification categories were determined using the ICE test method. Dr. Allen replied that the four-tiered EPA classification system was considered equivalent to the four-tiered GHS system and used the same ICE test method decision criteria (e.g., EPA Category I – GHS Category 1, EPA Category II = GHS Category 2A, EPA Category III = GHS Category 2B, EPA Category IV = GHS Category Not labeled).

Dr. Yu asked if the evaluation of the eyes was subjective and whether photographs were taken. Dr. Allen said that the evaluation of the eyes for corneal lesions was subjective, except for the measurement of corneal swelling, which is measured quantitatively using a pachymeter. He said that photographs were not typically taken but were recommended by the previous ocular Panel.

Dr. Merrill then presented the ICCVAM draft recommendations for the ICE test method for the Panel to consider.

Panel Evaluation

Dr. Tarlo (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the ICE test method and ICCVAM draft test method recommendations. The Panel agreed that the available data and test method performance supported the ICCVAM draft recommendations that the ICE test method is not recommended to identify substances from all hazard categories as defined by GHS, EPA, and EU classification systems. The Panel further agreed that the ICE test method is not recommended as a screening test to identify substances not labeled as irritants from all other hazard classifications defined by GHS, EPA, and EU, because one of the false negatives included a GHS Category 1 substance. The Panel agreed with the ICCVAM draft recommendation that the ICE test method should not be used as a screening test to identify GHS substances not labeled as irritants. Dr. van der Valk noted that the ICE test method is used by the Netherlands Organisation for Applied Scientific Research (TNO) to obtain good results, but the results obtained by other laboratories using the ICE test method in the validation study were variable. Dr. Vanparys recommended that the source of the variability be noted in the appropriate text.

The Panel agreed that the available data supported the ICCVAM draft recommendations that the proposed standardized protocol appeared acceptable. However, the Panel suggested that the protocol could be improved by adding objective endpoints for corneal opacity and fluorescein staining. The Panel also added that inclusion of a histopathological evaluation might improve ICE test method performance.

The Panel agreed with the ICCVAM draft recommendations for the ICE test method in terms of the proposed future studies that additional optimization studies would be required to validate the test method for the identification of all ocular irritancy hazard categories. The use of histopathology evaluation might add to the accuracy and determination of the test. The Panel also agreed with ICCVAM that the ICE test method performance standards are not warranted at this time.

Public Comments

Dr. Barroso said that variability of the ICE test method was similar to that of the Draize rabbit eye test because of the subjective assessments. He stated that the ICE test method should not be held to a higher standard than the Draize test. He also noted that the concordance among laboratories was reasonable.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion.

Isolated Rabbit Eye (IRE) Test Method

On behalf of NICEATM, Dr. Allen presented an overview of the IRE test method and reviewed the IRE draft BRD. Dr. Hayes asked whether the rabbits used by GlaxoSmithKline (GSK) were from PelFreeze Biologicals or if fresh eyes were used for each test. Dr. Allen replied that at least some of the rabbits were obtained from other GSK laboratories and had been used as negative controls from other acute safety testing. Dr. Ward noted that PelFreeze ships rabbit eyes from its facility in Rogers, Arkansas, adding that their rabbits are used for multiple purposes. She was not aware of a formal study to determine the acceptability of eyes shipped from the U.S. to Europe. Dr. Peiffer suggested

that shipped eyes should be carefully examined prior to use. Dr. Jester said that his laboratory has compared eyes obtained from an abattoir to fresh eyes and found no significant differences.

Dr. Merrill then presented the ICCVAM draft recommendations for the IRE test method for the Panel to consider.

Panel Evaluation

Dr. Tarlo (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the IRE test method and ICCVAM draft test method recommendations. The Panel agreed with ICCVAM that additional optimization and validation studies using a protocol that includes all four recommended endpoints are needed to further evaluate the relevance and reliability of the IRE test method and to develop more definitive recommendations.

The Panel recommended that the planned validation study with GSK/SafePharm include an evaluation of fresh versus shipped eyes. In general, the Panel felt there should be rigid criteria on the handling and storage of the eyes. Finally, the Panel recommended that criteria on test article administration/washout (e.g., viscous substances) were warranted.

Public Comments

No public comments were made.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion.

Bovine Corneal Opacity and Permeability Test Method (BCOP)

Dr. Curren, Institute for In Vitro Sciences, provided an overview of the BCOP test method. He noted that Pierre Gautheron and his colleagues initially developed the test method for occupational safety. Dr. Curren said that as many as 30% of bovine eyes are rejected upon inspection because of scratches and other defects, and emphasized the importance of including concurrent positive and negative controls in each study. With respect to histopathology evaluation, he said that it was important to carefully choose a qualified laboratory because of the impact of quality on the evaluation.

Dr. Vanparys pointed out that the 15x OD_{490} value in the *In Vitro* Score calculation was chosen to equate the data to *in vivo* data. One Panel member asked if there was an equilibration period, and Dr. Curren indicated that the bovine corneas were equilibrated for one hour before dosing.

Dr. Bailey asked if there was an example for when histopathology evaluation should be recommended based on effects associated with a particular chemical class. Dr. Curren cited as an example oxidizers, which may not produce opacity or permeability changes, but still produce substantive corneal damage that is observable only by histopathology. A Panel member asked why corneal thickness was not measured to provide a quantitative endpoint. Dr. Curren said that corneal thickness has been evaluated, but is less reliable than the opacity and permeability measurements and therefore is not measured in the current protocol.

Dr. Peiffer asked how the BCOP decision criteria for histopathology evaluation are applied to the EPA categorization scheme. Dr. Curren replied that a substance labeled as EPA Category IV would not penetrate further than the superficial corneal epithelium, whereas a Category III substance would penetrate to the basal layer, a Category II substance into the top third of the stroma, and a Category I substance into the bottom third of the stroma or to the endothelium. Minimal damage to the epithelium heals quickly, moderate damage heals more slowly, and significant damage (e.g., deep stromal or endothelial penetration) may be irreversible.

On behalf of NICEATM, Dr. Hamm reviewed the BCOP draft BRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the BCOP test method for the Panel to consider.

Panel Evaluation

Dr. Tarlo (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the BCOP test method and ICCVAM draft test method recommendations. With respect to the substances used in the validation studies, the Panel requested additional chemical classes be added as data becomes available to provide a more significant statistical inference. The Panel requested that Drs. Ahn and Palmer conduct a power analysis to determine the number of substances needed in each hazard classification to provide statistical significance.

The Panel discussed the performance of the BCOP test method to identify the intended range of classification categories. The Panel indicated that the available data and analyses were adequate for the intended purpose. The Panel indicated that all available and relevant data had been used in the ICCVAM BCOP test method analyses.

The Panel agreed with ICCVAM that the test method performance supported the ICCVAM draft recommendations. Accordingly, the BCOP test method was not recommended to identify substances from all hazard categories as defined by GHS, EPA, and EU classification systems. However, the BCOP test method can be used as a screening test to distinguish substances not labeled as irritants from all other hazard categories when results are to be used for EU or GHS hazard classifications. Because of the significant lesions associated with 50% (4/8) of the EPA Category III substances that tested as false negatives, the BCOP test method cannot be recommended as a screening test to identify EPA Category IV substances.

The Panel agreed with the ICCVAM draft recommendation that the BCOP test method could be used to distinguish substances not labeled as irritants from all other irritant classes, because the false negative rate for the EU and GHS systems was 0% (0/54 or 0/97, respectively). By comparison, the false negative rate was 6% (8/141) for the EPA system. Among the eight false negatives for the EPA system, 100% (8/8) were EPA Category III substances based on Draize rabbit eye test data.

The Panel said that, while the BCOP test method is unable to identify all irritant classifications, further test method development and refinement in future studies was encouraged.

The Panel recommended that performance standards should be developed, because the BCOP test method is now being considered as a screening test for both ocular corrosives/severe irritants and for the identification of substances not labeled as irritants.

Public Comments

Dr. Curren said that, based on his experience with the BCOP test method, performance of the BCOP for the four hazard classification systems was unlikely to improve based on the lack of Draize rabbit eye test reproducibility in the mild and moderate categories. He said that results from Weil and Scala (1971) show that the extremes are reproducible, but the mild and moderate levels of ocular irritation are highly variable. He referenced the antimicrobial cleaning products (AMCP) BRD that includes an analysis of the impact on the ocular hazard category when the results of a six-rabbit Draize test are randomly sampled for a three-rabbit test.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Harbell, Mary Kay Inc., said that his laboratories have used over 30,000 bovine eyes that were kept cold at 4ºC. He added that damaged eyes are quickly removed and excluded from the test. He pointed out that Gautheron et al. (1992) used both fresh eyes and eyes maintained at 4ºC and found no differences in their test method results. Dr. Harbell emphasized the utility of the BCOP in comparison to the other methods being considered given its focus on quantitative measurements.

Dr. Harbell also asked the Panel to consider how histopathology evaluation might contribute to the BCOP test method performance. He said that the experts at the 2005 ICCVAM workshop considered the depth of injury to be an important consideration in the assessment of ocular injury. The purpose of including histopathology evaluation is to evaluate the depth of injury that may not be visible to the naked eye. Dr. Harbell cited the example of oxidizing chemicals that may not affect the opacity or permeability of bovine eyes but do still damage the corneal tissue. Therefore, for these substances, depth-of-injury analysis may be important to differentiate corrosives or severe irritants from moderate irritants. Dr. Harbell said he would like to see histopathology evaluation reconsidered. Dr. Ward asked if he was recommending histopathology evaluation for all classes. Dr. Harbell said that he was but that it would be used primarily for EPA Categories I and II.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Barroso commented on what he referred to as the "top-down" (i.e., screening for corrosives/severe irritants) and "bottom-up" (i.e., screening for substances not labeled as irritants) approaches using the ICE and BCOP test methods. ECVAM is developing a paper to recommend the use of these proposed testing strategies for both ICE and BCOP, where substances could be tested in the BCOP or ICE test methods in order to identify corrosives/severe irritants or substances not labeled as irritants without using an animal test.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion (pending the results of a power analysis by Dr. Ahn) with one abstention, Dr. Vanparys, who cited a potential conflict-of-interest with the BCOP test method, which he had worked on at Johnson & Johnson.

Adjournment

After the discussion, Dr. Hayes adjourned the Panel for the day at 7:25 p.m., to reconvene at 8:30 a.m. on Thursday, May 21, 2009.

THURSDAY, MAY 21, 2009

Dr. Hayes convened the Panel at 8:30 a.m. and asked Dr. Stokes to discuss the conflict-of-interest for the day's planned topics. Dr. Stokes read the conflict-of-interest statement and Dr. Hayes asked the Panel to declare any conflicts-of-interest. The conflicts-of-interest declared by Panel members on day one of the meeting were repeated.

Dr. Hayes then asked for introductions from the Panel, NICEATM staff, members of ICCVAM and the OTWG, and those in attendance for the public session.

The first order of business was to address issues from the preceding day.

BCOP Power Calculation

Dr. Ahn reported on the power calculation requested on Wednesday May 20, 2009, for the BCOP test method. He determined that, for each of the four hazard classification systems, a sample size of 13 substances in each chemical class represented (i.e., 13 x 4 for each chemical class for a four-category hazard classification system) is required to achieve 80% power using a two-group normal approximation test for proportions with a one-sided 0.05 significance level. This is necessary to reject the null hypothesis that the BCOP test is inferior to the Draize rabbit eye test (the accuracy of the BCOP test is more than 0.1 less than that of the Draize test) in favor of the alternative hypothesis that the accuracies in the two groups are equivalent. Dr. Ahn also noted that his analysis included the assumption that the expected accuracy of the BCOP test is 0.6 and the expected accuracy of the Draize rabbit eye test is 0.9.

The Panel voted unanimously to include the recommendation that a sample size of 13 be used for each chemical class in each of the four hazard classifications to achieve statistical significance.

ICE Test Method False Negative Substances

Dr. Vanparys commented on the ability of the ICE test method to identify GHS substances not labeled as irritants. Dr. Vanparys indicated that the false negative substances listed in the ICCVAM BRD were either paints that stick to the cornea or solids, which are known to give inaccurate results with the ICE test method. Dr. Vanparys suggested that the ICE test method is capable of identifying GHS substances not labeled as irritants with the exception of solids and substances that stick to the cornea. The overall Panel recommendations, as stated the previous day, remained unchanged.

Low Volume Eye Test (LVET) Test Method

On behalf of NICEATM, Dr. Allen provided a brief overview of the LVET test method and reviewed the LVET draft SRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the LVET for the Panel to consider.

Panel Evaluation

Dr. Sawyer (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the LVET and ICCVAM draft test method recommendations. The Panel noted that the LVET has been used on a wide range of substances and that it does detect the full range of ocular irritancy, but recognized that the majority of the LVET database was for surfactants and surfactant-containing products. The Panel identified several references that should be added to the SRD and noted the need to review the ECVAM BRD. If any additional historical data were obtained, there might be sufficient data to determine the performance of the LVET on several other chemical classes.

The Panel indicated that pain associated with direct application of the test substance to the cornea should not be an issue in light of the recommendations for topical anesthetic and systemic analgesic use.

When discussing the performance of the LVET compared to the Draize test, the Panel indicated that the evaluation was adequate, noting that the LVET appeared to overpredict the human response to a lesser degree than the Draize rabbit eye test. They also recommended that the full range of irritation categories are represented in the LVET validation database.

In considering whether all available data had been made available, the Panel indicated that all data had not been evaluated. Additional published sources should be considered as well as the ECVAM BRD, on which the Panel was unable to comment during this meeting. The Panel stated that in the absence of all existing data, including a background review document prepared by the European Centre for the Validation of Alternative Methods, it could not make definitive conclusions or recommendations on the validation status of the LVET. Nonetheless, the Panel did consider the limited data that are available for the LVET to support the use of historical LVET data as acceptable *in vivo* reference data on which to base comparisons to *in vitro* study results.

Public Comments

Dr. Harbell commented that eye irritation testing is done to protect the public and that accidental exposure data should be included in the evaluation. Dr. Harbell also commented on Dr. Merrill's presentation that outlined the ICCVAM draft recommendations. He stated that the suggestion in the ICCVAM draft recommendations that severe substances should be tested in humans is terrifying. (Note: This comment was in response to a misinterpretation by the commenter, which was clarified by Dr. Merrill who stated that the ICCVAM draft recommendations do not recommend human testing to be conducted [see below]).

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Curren commented that the LVET is being discussed because it was used as an *in vivo* reference test method for some of the data provided for the antimicrobial cleaning product (AMCP) testing strategy. He stated that only biologic or LVET data exist for many of the AMCPs, and these data were used to determine the prediction model to support registration of these AMCPs. The LVET test method is no longer used, but there is historical data that can and should be used. Dr. Curren stated that the question is whether we are putting people at risk based upon the cut-off points suggested in the AMCP BRD.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. McNamee (Procter & Gamble) reiterated the comments by Dr. Curren regarding the LVET and noted that 30 years of human experience data with a chemical substance are sufficient for licensing in the United Kingdom.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Merrill responded to the comment by Dr. Harbell regarding human testing. Dr. Merrill clarified that the ICCVAM draft recommendation states that if an organization or sponsor desires to more adequately characterize the usefulness and limitations of the LVET, ICCVAM recommends that a comprehensive set of substances be tested and compared with the Draize rabbit eye test results. She stated that there was no recommendation for human testing to be conducted, but that existing accidental human injury data and ethical human study data should always be considered.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion with one abstention,

Dr. Ward, who cited a potential conflict-of-interest because of her previous consulting work for a company that conducts the LVET.

Cytosensor® Microphysiometer Test Method

Dr. Curren provided an overview of the Cytosensor Microphysiometer (CM) test method protocol.

On behalf of NICEATM, Dr. Lipscomb reviewed the CM test method performance as detailed in the AMCP draft SRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the CM test method for the Panel to consider.

Panel Evaluation

Dr. Bailey (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the CM test method and ICCVAM draft test method recommendations. The Panel indicated that the test method protocol was sufficiently detailed; however, it was unlikely to be widely used because the CM instrument has been discontinued and a new instrument would require revalidation.

The Panel recommended the use of relevant positive controls in any future validation studies and, because surfactants form micelles that can influence response, surfactant concentrations should be included. The Panel recommended that an evaluation of the different classes of surfactants (i.e., nonionic, anionic, cationic, and zwitterionic) be conducted to determine if restrictions should be imposed on use of the CM test method.

The Panel agreed that, based on the database of surfactants and surfactant-based formulations, LVET data could be used to support the validity of the CM test method in the proposed AMCP testing strategy.

The Panel also agreed that the additional data on the surfactants and surfactant-containing formulations in the ECVAM BRD provided sufficient support for the use of the CM test method as a screening test to identify water-soluble surfactant chemicals and certain types of surfactant-containing formulations (e.g., cosmetics and personal care product formulations but not pesticide formulations) as either severe or corrosive irritants or substances not labeled as irritants in a tiered-testing strategy, as part of a weight-of-evidence approach. The Panel also agreed that the intra- and interlaboratory reproducibility of the CM test method had been adequately evaluated, although for a limited range of substances as previously discussed. The Panel again noted that the instrument has been discontinued and is currently not supported by the manufacturer, making its use difficult. However, if the CM instrument were redesigned, the remanufactured instrument would require "catch-up" validation (i.e., not a full validation study).

Based upon the lesions noted for one false negative substance in the EPA classification system, the Panel expressed concern with the ability of the CM test method to identify EPA Category IV substances. The Panel noted that the rabbit data indicated that this substance would be classified as a Category III and, therefore, may cause irritation in a human. The Panel noted that further CM studies are needed, in particular for EPA Categories III and IV substances.

The Panel also expressed concern with the high false positive rate of the CM test method when identifying all four hazard categories.

Public Comments

Dr. Curren noted a correction to his presentation where he did not specifically state that the CM test method is limited to water-soluble substances. He questioned the need for performance standards for the CM test method, given that the Panel did not recommend performance standards for the BCOP

and ICE test methods. Dr. Curren commented that the surfactants referred to as *personal care products* are really detergents.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion.

EpiOcular Test Method

Dr. Curren provided an overview of the EpiOcular (EO) test method protocol.

On behalf of NICEATM, Dr. Lipscomb reviewed the EO test method performance as detailed in the AMCP draft SRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the EO test method for the Panel to consider.

Panel Evaluation

Dr. Bailey (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the EO test method and ICCVAM draft test method recommendations. The Panel agreed that the EO test method protocol is adequately detailed but emphasized that the manufacturer should provide a "certificate of quality" for each batch of EO. The Panel also agreed that the critical aspects of the protocol had been justified and described in the BRD; however, in order to use the EO test method in a testing strategy to identify mild irritants and substances not labeled as irritants, positive controls that represent these hazard categories should be included in any future validation studies. The Panel noted that the EO test method cannot distinguish Category III from Category IV substances.

The Panel commented that the performance of the EO test method had not been adequately evaluated and compared to the Draize test for the types of substances included in the AMCP database. The Panel noted that the total number of products and their distribution across hazard categories were not sufficient. The Panel commented that the intralaboratory variability was not adequately assessed, although interlaboratory variability was considered to be adequate.

Public Comments

Dr. Curren indicated that he felt that it was appropriate to include EO data that used a different protocol as a measure of test method reproducibility.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion with one abstention, Dr. Ward, who cited a potential conflict-of-interest because of her previous consulting work for a company that conducts the EO test method.

Strategy for U.S. Environmental Protection Agency Ocular Hazard Classification and Labeling of Antimicrobial Cleaning Products (AMCPs) Using *In Vitro* Alternative Test Methods

Dr. Curren provided an overview of the AMCP testing strategy.

On behalf of NICEATM, Dr. Lipscomb reviewed the AMCP draft SRD.

Dr. Merrill then presented the ICCVAM draft recommendations for the AMCP testing strategies for the Panel to consider.

Panel Evaluation

Dr. Bailey (Evaluation Group Chair) presented the Evaluation Group's responses to questions posed to the Panel on the validation status of the AMCP testing strategies and ICCVAM draft test method recommendations. The Panel also suggested adding more discussion of the cells used in the CM and EO test methods.

Regarding the BCOP test method, the Panel reflected on its previous discussions of the BCOP test method for the total database. The Panel indicated that use of the BCOP test method in a testing strategy to identify severe irritants (Category I) and moderate irritants (Category II), should include positive controls that represent these hazard categories in any future validation studies. The Panel noted that histopathology evaluation, as it is proposed at this time as an additional endpoint for the BCOP test method, does not justify its use for hazard classification of AMCPs. However, histopathology evaluation may prove to be a useful endpoint and, as such, collection of histopathology data and further efforts to optimize its use are encouraged.

The Panel agreed with the ICCVAM draft recommendations that there is insufficient data to support the testing strategy in terms of the proposed test method usefulness and limitations (i.e., the classification of substances in all four ocular hazard categories). There were also insufficient available data on which to base definitive recommendations on the proposed alternate testing strategy for classifying substances in all four ocular hazard categories. In discussing the validity of retrospective evaluations, the Panel stated that a retrospective evaluation of results could be considered adequate if the studies were performed with GLP compliance, coded samples, and pre-established evaluation criteria. The Panel commented that any definitive recommendations on a testing strategy should be based on prospective testing of a list of reference substances in each of the proposed *in vitro* test methods.

The Panel concurred with the ICCVAM draft recommendations in terms of the proposed test method standardized protocols. The Panel stated that routine fixation of tissue from the BCOP test method for possible histopathology evaluation should be continued. The Panel emphasized that no single *in vitro* test method alone was applicable to all types of test materials, and therefore suggested several future studies that could potentially expand the usefulness of AMCP test strategies.

Finally, the Panel commented that the development of performance standards for the AMCP testing strategy was not currently warranted and that a new approach needed to be defined for comparing testing strategies.

Public Comments

Dr. Barroso commented that ECVAM is working on a guideline for the detection of severe irritants with the BCOP test method. He indicated that they see a small change in classification when the cut-off is changed from 55 to 75. ECVAM considers 55 the best cut-off for their intended purpose.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Dr. Curren commented that concern regarding the limited number of AMCPs is misplaced due to the intended narrow applicability domain. He stated that industrial-strength cleaners are mostly severe irritants and that household cleaners are mostly mild irritants. Very few, if any, substances are in the moderate range. Dr. Curren expressed concern with the recommendation by the Panel that substances need to be tested by each test method in the testing strategy. He noted that histopathology evaluation with the BCOP test method was included in the testing strategy to provide additional safety, and clarified that most of the histopathology evaluation was performed by a certified veterinary

pathologist. He also questioned the Panel's suggested use of a transformed ocular cell line rather than a normal epidermal cell line.

Dr. Hayes asked the Panel if they needed clarification from the commenter; none were requested.

Panel Conclusions and Recommendations

Dr. Hayes asked if the Panel was in agreement with its preliminary conclusions. The Panel voted unanimously to approve the recommendations as revised during the discussion with one abstention, Dr. Ward, who cited a potential conflict-of-interest because of her previous consulting work for a company that manufactures AMCPs.

Concluding Remarks

Dr. Hayes, on behalf of the Panel, thanked Dr. Stokes and the NICEATM staff for their continued assistance during the review process and Panel meeting. He also thanked Dr. Wind, ICCVAM Chair, and the members of ICCVAM and the OTWG for their contributions to the project. Finally, Dr. Hayes thanked the Panel and the Evaluation Group Chairs.

Drs. Wind and Stokes thanked the Panel again for their hard work, thoughtful and objective deliberations, and advice. Dr. Stokes further thanked public attendees for their participation and the invited test method developers for their excellent test method summaries. Dr. Stokes concluded by saying he looked forward to working further with Panel members to complete the Panel report.

Adjournment

Dr. Hayes adjourned the Panel at 7:40 p.m., concluding the meeting.